GENOTOXICITY ASSESSMENT OF NANO-PARTICLES ON MICROPROPAGATED OLIVE (OLEA EUROPAEA L.) PLANTS USING RAPD AND DAMD MARKERS

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Abstract

Nanomaterials have been widely used to improve the plant’s growth in vitro. The aim of the current study was to evaluate positive and negative effects of nanotechnology in plant tissue culture by treatment of three olive cultivars ‘Koroneiki’, ‘Picual’ and ‘Manzanillo’ with different concentrations of silver or selenium nanoparticles(NPs), compared with the control. In addition, genotoxicity evaluation of nanoparticles on olive plants by molecular markers. Effect of AgNPs or SeNPs on genomic DNA was studied by Random amplified polymorphic DNA (RAPD) and Direct amplification of mini satellite-region DNA(DAMD) profiles, which allow detection of alterations in DNA sequence between treated and untreated olive plants. Differences in RAPD and DAMD assays were analyzed depending on the appearance or disappearance of bands. Amplification profiles of genomic DNA with molecular markers indicating that olive plantlets treated with (2.5 and 5 mg L⁻¹ SeNPs) and (5 and 10 mg L⁻¹ AgNPs) scored polymorphism which ranged from 41.10 to 41.46% using RAPD and DAMD techniques, respectively. This study is the first report stated that 5 mg L⁻¹ AgNPs are safe concentration for olive growth in vitro. In contrast, the high concentrations of AgNPs (10 mg L⁻¹) and SeNPs (2.5 and 5 mg L⁻¹) induced DNA change of olive plants which confirms the NPs as a potential environmental hazard.

Key words: Tissue cultures, silver nanoparticles, selenium nanoparticles, cluster analysis.

Introduction

The olive tree (Olea europaea L.) is one of the most important fruit crops in the Mediterranean basin (Fabbri et al., 2004), olive trees were asexual propagated by cuttings and grafting (Hartmann and Kester, 1975). Plant tissue cultures is a powerful technique which permits fast clonal propagation, production of pathogen-free plants, under controlled conditions and helps in the genetic improvement of crops (Vasil, 1988; Zucherrelli and Zucherrelli, 2002; Mangal et al., 2014; Abdallatif et al., 2015). Nanotechnology, a novel emerging field of science, allows advanced study and new applications in the field of agriculture and biotechnology (Sobha et al., 2010; Siddiqui et al., 2015). Nanoparticles (NPs) are produced in the size range of 1-100 nm (Roco, 2003). Nanomaterials have been broadly applied to enhance germination of seed, improve the growth of plants, yield and bioactive component content (Wang et al., 2016; Ruttkay-Nedecky et al., 2017). NPs have been confirmed to be advantageous for various contamination kinds elimination (Sondi and Salopek-Sondi, 2004; Jo et al., 2009; Wang et al., 2017; Spinoso-Castillo et al., 2017). Several reports have proven positive effects of NPs on thecallus induction, shoot multiplication and the plants growth promote in vitro (Al-Khayri and Al-Bahrany, 2001; Kumar et al., 2009; Aghdae et al., 2012). AgNPs treatment increased survival and delayed explants senescence (Sarmast et al., 2015). Sharma et al., (2012) reported that incorporation of silver nanoparticles at 50 mg L⁻¹ in MS...
basal medium enhanced the growth characteristics of mustard (*Brassica juncea*) plantlets by reducing the content of malondialdehyde (MDA), hydrogen peroxide (H$_2$O$_2$) and proline by activating antioxidant isozymes. The majority of nanomaterials cause toxicity at some concentrations by changing plant morpho-anatomical, physiological, biochemical and genetic constitutions (Puaand Chi, 1993; Rico et al., 2015). Several reports have indicated the phytotoxicity of NPs used at higher concentrations to plant tissues (Craig and Jason, 2011; Ghodake et al., 2011). NPs affect the mitotic activity and change the DNA profile and gene expression in the plants (Athva et al., 2012; Landa et al., 2015; Ewais et al., 2015; Tripathi et al., 2017). NPs phytotoxicity can be caused by the generation of reactive oxygen species (ROS), which results in lipid peroxidation, proteins and DNA damage (Arruda et al., 2015; Maet al., 2015). Kokina et al., (2017) investigated the effect of AgNPs on somaclonal variation in the flax, somaclonal variation was higher in both callus and regenerated explants grown on medium containing NPs. López-Moreno et al., (2010) observed damage in the DNA structure in soybean plants in the presence of 2000 and 4000 mg/L CeO$_2$ NPs. A similar effect was observed in the onion and tobacco (Ghosh et al., 2010) squash (Moreno-Olivas et al., 2014) and maize (Castiglione et al., 2011). Moreover, negative impacts were also shown in chromosome structures of maize (Castiglione et al., 2011). These kinds of damaging effects have been obtained by Random amplified polymorphic DNA (RAPD) technique, which uses to characterize and verify the origin, stability of clones and plants micropropagated by tissue culture technique (Pijut et al., 2007). The aim of the current study was to evaluate positive and negative effects of nanotechnology in olive plants micropropagated *in vitro* by treatment of three olive cultivars ‘Koroneiki’, ‘Picual’ and ‘Manzanillo’ with two different concentrations of silver or selenium nanoparticles, compared with the control. In addition, evaluation of genotoxicity for treating plants with NPs by RAPD and DAMD profiles.

**Materials and Methods**

**Plant materials and explants preparation**

The current research was carried out during (2018/19) seasons at the laboratory of Pomology Department, Faculty of Agriculture, Cairo University and Tissue culture technique Lab. Central Laboratories Network, Pomology Dept., and Genetic Engineering and Biotechnology Research Division, Genetics and Cytology Department, National Research Centre. Active spring shoots were collected from mature olive trees of cultivars namely ‘Koroneiki’, ‘Picual’ and ‘Manzanillo’, during the summer season. After removing of leaves; the shoots were cut into nodal segments. Surface sterilization performed according to Hassan et al., (2017).

**In vitro propagation**

Nodal segments of the selected olive cultivars were cultured on Rugini olive medium (Rugini, 1984) supplemented with 2 mg L$^{-1}$ of zeatin. Two types of nanoparticles were used silver nanoparticles (AgNPs) at concentrations of 5 and 10 mg L$^{-1}$ and selenium nanoparticles (SeNPs) at concentrations of 2.5 and 5 mg L$^{-1}$. All media were supplemented with 30 g L$^{-1}$ mannitol and 6 g agar L$^{-1}$ and autoclaved at 121°C for 15 min. Four explants were cultured on 50 ml of semi-solid medium and maintained in the growth chamber at 23-25°C in 16/8 h (light/dark), with 40-60μmol m$^{-2}$s$^{-1}$ provided by cool-white fluorescent lamps. After three weeks the sprouted buds were transferred to fresh media of the same composition and the sub-culture was performed every four weeks. Scores were given for necrosis and development as follows: Negative results = 1; below average = 2; average = 3, above average = 4 and excellent = 5 according to Pottino (1981). Treatments were arranged in a Completely randomized design. Each treatment was replicated three times, according to Snedecor and Cochrone (1980). The obtained data were statistically analyzed and the means were difference according to Duncan multiple range test 1% level (Duncan, 1955).

**Extraction of genomic DNA**

0.5 g of fresh young olive leaves from the studied three cultivars (‘Koroneiki’, ‘Picual’ and ‘Manzanillo’) collected from silver (Ag) and selenium (Se) nanoparticles treated and untreated olive plants and were soaked in liquid nitrogen for DNA extraction using the 2% Cetyltrimethyl ammonium bromide (CTAB) procedure as described by Murry and Thompson, (1980) with modification by De la Rosa et al., (2002).

**RAPD and DAMD profiles**

Random amplified polymorphic DNA (RAPD) and Direct amplification of mini satellite-region DNA (DAMD) techniques of NPs treated and untreated olive plants were performed, using six RAPD and four DAMD primers as shown in Table (3) (Life Technologies, Gaithersburg, Md.). Each 25-μl amplification reaction containing 1X PCR reaction buffer, 2.0 mM MgCl$_2$, 0.2 mM dNTPs mix, 0.4 μM of each primer set, 1.5 U Taq DNA polymerase and adjusted to 25 μl using ddH$_2$O. Amplifications were carried out in DNA a thermo-cycler (Biometra, Germany) programmed as follows: 1 initial
denaturation step at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 36°C for 1 min and 72°C for 1 min, and a final extension cycle at 72°C for 10 min.

**Amplification product analysis**

Amplification products were separated on a 1.5% agarose gel containing 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) at 90 V. The genomic DNA was stained with RedSafe Nucleic Acid Staining Solution (1/20,000) (iNtRON Biotechnology, Inc. Kr). The gels were analyzed by UVI Geltec version 12.4, 1999-2005 (USA).

**Data analysis**

A matrix for RAPD and DAMD combined was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the cultivar. Genetic similarity coefficients were computed according to Nei and Li (1979). The data were subsequently used to construct a dendrogram using the un-weighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973) employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). All the computations were carried out using the software NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.02 (Rohlf, 2000). Jaccard’s coefficients were calculated using similarity coefficients obtained from combined RAPD and DAMA profiles.

**Results and Discussion**

**Effect of nanoparticles on different olive cultivars**

Data in tables 1 and 2 shows the effect of using nanoparticle type and concentration on shoots of different olive cultivars at the proliferation stage. It was obtained
that the addition of nanoparticle to the culture medium had a positive effect of olive explant compared with the control. They significantly reduced necrosis in the three olive cultivars and improved growth vigor and development of the cultured olive explants. AgNPs at 5 mg L$^{-1}$ recorded the highest value of explant development, followed by AgNPs at 10 mg L$^{-1}$, while the control recorded the lowest value (fig. 1). Regarding the effect of nanoparticle on necrosis, AgNPs at both concentrations recorded the lowest value of explant necrosis, followed by SeNPs, while the control recorded the highest value. Several reports have proven positive effects of NPs on the shoot multiplication and the plant’s growth in vitro (Aghdaei et al., 2012; Kumar et al., 2009). Silver nitrate (AgNO$_3$) nanoparticles treatment increased survival and delayed explants senescence (Sarmast et al., 2015). AgNO$_3$ has shown to have important effects in plant tissue culture, improving micropropagation (Kotsias and Roussos, 2001; Naik and Chand, 2003) in many species. The inhibition of ethylene action or its production using AgNO$_3$ has shown stimulatory effects on plant regeneration (Palmer, 1992). The silver ion, a potent inhibitor of ethylene action (Songstad et al., 1988) has been reported to stimulate shoot regeneration in wheat and tobacco (Beyer, 1976) or in maize tissue culture (Purnhauser et al., 1987). Vannini et al. (2014) observed a toxic effect of 10 mg L$^{-1}$ AgNPs on wheat seedlings at the early growth.

**RAPD assay**

Six decamer RAPD primers were used to study genotoxicity effect of AgNPs and SeNPs on three olive cultivars regenerated in vitro compared with untreated ones (fig. 2 and table 3). A total of 73 scorable bands, ranging from 100 (RAPD-4) to 2000bp (RAPD-2) were indicated using the six RAPD primers. The number of amplified products per primer varied from nine (RAPD-5 and RAPD-6) to 21 (RAPD-2). Forty-three bands out of the 73 fragments were monomorphic (58.90%) and 30 reproducible bands were polymorphic (41.10%). The RAPD-5 recorded the highest polymorphism with 66.67% (table 3). In contrast, RAPD-3 and RAPD-6 exhibited the lowest polymorphism (33.33%). Three out of the 73 were bands-specific (4.11%) (table 3). The olive plantlets cv ‘Koroneiki’ treated with 2.5 mg/L SeNPs scored one positive marker of +700 bp, using primer RAPD-4. Moreover, ‘Koroneiki’ and ‘Picual’ cultivars treated with 5 mg/L SeNPs revealed one allele of +590 bp, using primer RAPD-2. Furthermore, the control plantlets cv ‘Koroneiki’ recorded one amplicon of +300 bp, using primer RAPD-2 and absent in the other controls and treatments (table 3).

**DAMD profiles**

Four DAMD primers amplified genomic DNA extracted of NPs treated and untreated olive plantlets. A number total of 41 alleles were recorded using four primers DAMD (fig. 3 and table 4). The number of amplicons varied from 100bp (primer DAMD-3) to 1500bp (primer DAMD-4). Twenty-four out of 41 were monomorphic (58.54%), 17 amplified fragments were polymorphic (41.46%). The maximum number of loci was showed in primers DAMD-2, DAMD-3 and DAMD-4 (11 loci), while the minimum number of loci was observed in primer DAMD-1 (eight bands). The DAMD-4 scored

<p>| Table 1: The effect of nanoparticle type and concentration on necrosis of different olive cultivars. |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cultivar</th>
<th>Manzanillo</th>
<th>Picual</th>
<th>Koroneiki</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>5 mg L$^{-1}$ Silver</td>
<td></td>
<td>2.89</td>
<td>1.50</td>
<td>2.67</td>
</tr>
<tr>
<td>10 mg L$^{-1}$ Silver</td>
<td></td>
<td>2.44</td>
<td>1.39</td>
<td>2.22</td>
</tr>
<tr>
<td>2.5 mg L$^{-1}$ Selenium</td>
<td></td>
<td>1.89</td>
<td>1.20</td>
<td>1.89</td>
</tr>
<tr>
<td>5 mg L$^{-1}$ Selenium</td>
<td></td>
<td>1.49</td>
<td>1.00</td>
<td>1.56</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2.54</td>
<td>2.46</td>
<td>1.82</td>
</tr>
<tr>
<td>LSD of treatment</td>
<td></td>
<td>0.167</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD of cultivars</td>
<td></td>
<td>0.129</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD interaction</td>
<td></td>
<td>0.289</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table 2: The effect of nanoparticle type and concentration on development of different olive cultivars. |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cultivar</th>
<th>Manzanillo</th>
<th>Picual</th>
<th>Koroneiki</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>56.00</td>
<td>56.00</td>
<td>56.00</td>
</tr>
<tr>
<td>5 mg L$^{-1}$ Silver</td>
<td></td>
<td>60.67</td>
<td>53.00</td>
<td>70.33</td>
</tr>
<tr>
<td>10 mg L$^{-1}$ Silver</td>
<td></td>
<td>70.00</td>
<td>61.00</td>
<td>82.00</td>
</tr>
<tr>
<td>2.5 mg L$^{-1}$ Selenium</td>
<td></td>
<td>94.00</td>
<td>65.33</td>
<td>97.00</td>
</tr>
<tr>
<td>5 mg L$^{-1}$ Selenium</td>
<td></td>
<td>82.67</td>
<td>70.00</td>
<td>94.33</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>72.67</td>
<td>61.07</td>
<td>79.93</td>
</tr>
<tr>
<td>LSD of treatment</td>
<td></td>
<td>1.803</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD of cultivars</td>
<td></td>
<td>1.397</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD interaction</td>
<td></td>
<td>3.120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Genotoxicity Assessment of Nano-particles on Micropropagated Olive

Fig. 2: RAPD-PCR analysis of three olives cultivars treated with selenium and silver nanoparticles. Lane M = 100 bp DNA ladder. Lane 1 = ('Picual', control); lane 2 = ('Picual', 2.5 mg/L SeNPs); lane 3 = ('Picual', 5 mg/L SeNPs), lane 4 = ('Picual', 5 mg/L AgNPs); lane 5 = ('Picual', 10 mg/L AgNPs); lane 6 = ('Manzanillo', control), lane 7 = ('Manzanillo', 2.5 mg/L SeNPs); lane 8 = ('Manzanillo', 5 mg/L SeNPs); lane 9 = ('Manzanillo', 5 mg/L AgNPs); lane 10 = ('Manzanillo', 10 mg/L AgNPs); lane 11 = ('Koroneiki', control); lane 12 = ('Koroneiki', 2.5 mg/L SeNPs); lane 13 = ('Koroneiki', 5 mg/L SeNPs), lane 14 = ('Koroneiki', 5 mg/L AgNPs) and lane 15 = ('Koroneiki', 10 mg/L AgNPs).

Fig. 3: DAMD profiles of three olives cultivars treated with selenium and silver nanoparticles. Lane M = 100 bp DNA ladder. Lane 1 = ('Picual', control); lane 2 = ('Picual', 2.5 mg/L SeNPs); lane 3 = ('Picual', 5 mg/L SeNPs), lane 4 = ('Picual', 5 mg/L AgNPs); lane 5 = ('Picual', 10 mg/L AgNPs); lane 6 = ('Manzanillo', control), lane 7 = ('Manzanillo', 2.5 mg/L SeNPs); lane 8 = ('Manzanillo', 5 mg/L SeNPs); lane 9 = ('Manzanillo', 5 mg/L AgNPs); lane 10 = ('Manzanillo', 10 mg/L AgNPs); lane 11 = ('Koroneiki', control); lane 12 = ('Koroneiki', 2.5 mg/L SeNPs); lane 13 = ('Koroneiki', 5 mg/L SeNPs), lane 14 = ('Koroneiki', 5 mg/L AgNPs) and lane 15 = ('Koroneiki', 10 mg/L AgNPs).
Fig. 4: Dendrogram of three olives cultivars treated with selenium and silver nanoparticles depend on combined RAPD and DAMD by UPGMA cluster analysis from the similarity matrix obtained. K= ‘Koroneiki’, M= ‘Manzanillo’ and P= ‘Picual’.

Table 3: RAPD-PCR analysis of three olives cultivars treated and untreated with silver and selenium nanoparticles.

<table>
<thead>
<tr>
<th>Primer Code No.</th>
<th>Primer sequences</th>
<th>Size range of the scorable loci (bp)</th>
<th>Total loci</th>
<th>No. of monomorphic loci</th>
<th>No. of polymorphic loci</th>
<th>% polymorphism</th>
<th>Unique loci</th>
<th>Molecular size of markers (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAPD-1</td>
<td>CAGGCCCTTTC</td>
<td>130-1200</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>45.45%</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>RAPD-2</td>
<td>TGCCGAGCTG</td>
<td>120-2000</td>
<td>21</td>
<td>13</td>
<td>8</td>
<td>38.10%</td>
<td>2</td>
<td>+300;+590</td>
</tr>
<tr>
<td>RAPD-3</td>
<td>CCCGTCAGCA</td>
<td>156-1310</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td>33.33%</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>RAPD-4</td>
<td>GGGTACGCAGCC</td>
<td>100-820</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>36.36%</td>
<td>1</td>
<td>+700</td>
</tr>
<tr>
<td>RAPD-5</td>
<td>GTGTCGCGAG</td>
<td>285-920</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>66.67%</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>RAPD-6</td>
<td>CAGCAACCACC</td>
<td>280-1420</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>33.33%</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>100-2000</td>
<td>73</td>
<td>43(58.90%)</td>
<td>30(41.10%)</td>
<td>41.10%</td>
<td>3</td>
<td>4.11%</td>
</tr>
</tbody>
</table>

Table 4: DAMD analysis of three olives cultivars untreated and treated with silver and selenium nanoparticles.

<table>
<thead>
<tr>
<th>Primer Code no.</th>
<th>Primer sequences</th>
<th>Size range of the scorable loci (bp)</th>
<th>Total loci</th>
<th>No. of monomorphic loci</th>
<th>No. of polymorphic loci</th>
<th>% polymorphism</th>
<th>Unique loci</th>
<th>Molecular size of markers (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAMD-1</td>
<td>GGTGTAGAGAGGGGT</td>
<td>155-1330</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>37.50%</td>
<td>2</td>
<td>-1250;+155</td>
</tr>
<tr>
<td>DAMD-2</td>
<td>CCTCCTCCCTCCT</td>
<td>169-1270</td>
<td>11</td>
<td>9</td>
<td>2</td>
<td>18.18%</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>DAMD-3</td>
<td>GAGGTTTTTCCA</td>
<td>100-1450</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>45.45%</td>
<td>2</td>
<td>+100;+1000</td>
</tr>
<tr>
<td>DAMD-4</td>
<td>GAGGGTGCCCGTTCT</td>
<td>129-1500</td>
<td>11</td>
<td>4</td>
<td>7</td>
<td>63.64%</td>
<td>3</td>
<td>+129;810;+1451</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>100-1500</td>
<td>41</td>
<td>24(58.54%)</td>
<td>17</td>
<td>41.46%</td>
<td>7</td>
<td>17.07%</td>
</tr>
</tbody>
</table>
the highest polymorphism with 63.64%, followed by primer DAMD-3 (45.45%). On the contrary, primer DAMD-2 exhibited the lowest polymorphism (18.18%). Seven out of the 41 were markers (17.07%) (Table 4). The control plantlet cv 'Picual' appeared two negative markers of -1250 and -810 bp, using DAMD-1 and DAMD-4, respectively which revealed in all other treatments. In addition, 'Picual' control induced four positive markers with molecular sizes (+100 and +1000bp), (+129 bp), (+155bp), using primers DAMD-3, DAMD-4 and DAMD-1, respectively. Besides, 'Picual' plantlets treated with 2.5 mg/L SeNPs showed one specific band with molecular size +1451 bp, using primer DAMD-4 (Table 4).

In the current study, RAPD and DAMD profiles of screening DNA change, induced by non-lethal levels of olive plantlets subject to different concentrations of Ag and Se NPs. Differences in RAPD and DAMD assays were analyzed depending on the appearance or disappearance of bands. Amplification profiles of genomic DNA with molecular markers indicating that olive plantlets treated with 5 and 10 mg L\textsuperscript{-1} AgNPs and 2.5 and 5 mg L\textsuperscript{-1} SeNPs scored polymorphism, which ranged from 41.10 to 41.46% using RAPD and DAMD assays, respectively. Novel fragments were obtained when genomic DNA amplified from the olive plants treated with nanoparticles based on the nanomaterial concentration and primer. These results were in agreement with Singh et al. (2009) found that RAPD-PCR profiles supply a simple and fast way to study genotoxicity on onion plants, compared with conventional ways, this can be an analysis of a big number of plants through short time. It can be concluded from the study that 10 mg L\textsuperscript{-1} NO\textsubscript{3}\textsuperscript{-} NOGP. causes DNA damage by modification and oxidation on DNA, double-strand breaks, DNA-protein cross-linking, point mutation, bulky adducts, altered gene expression and an introduction of basic sites, all of which affect DNA amplification and primer binding in RAPD-PCR analysis. The NPs are shown to produce genotoxicity involve metal/metal-oxide NPs such as AgO, CuO, and NiO nanoparticles.

Table 5. Similarity index among the three olives cultivars treated with silver and selenium nanoparticles by combined RAPD and DAMD.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>P (control)</th>
<th>Picual (2.5 mg/L SeNPs)</th>
<th>Picual (5 mg/L Ag NPs)</th>
<th>Manzanillo (2.5 mg/L SeNPs)</th>
<th>Manzanillo (10 mg/L Ag NPs)</th>
<th>Koroneiki (control)</th>
<th>Koroneiki (2.5 mg/L SeNPs)</th>
<th>Koroneiki (5 mg/L Ag NPs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picual</td>
<td>(control)</td>
<td>1.00</td>
<td>0.81</td>
<td>0.75</td>
<td>0.79</td>
<td>0.80</td>
<td>0.76</td>
<td>0.73</td>
<td>0.79</td>
</tr>
<tr>
<td>Picual</td>
<td>(5 mg/L SeNPs)</td>
<td>0.81</td>
<td>0.75</td>
<td>0.79</td>
<td>0.80</td>
<td>0.76</td>
<td>0.73</td>
<td>0.78</td>
<td>0.79</td>
</tr>
<tr>
<td>Picual</td>
<td>(5 mg/L Ag NPs)</td>
<td>0.75</td>
<td>0.79</td>
<td>0.80</td>
<td>0.76</td>
<td>0.73</td>
<td>0.78</td>
<td>0.79</td>
<td>0.79</td>
</tr>
<tr>
<td>Manzanillo</td>
<td>(control)</td>
<td>0.76</td>
<td>0.84</td>
<td>0.85</td>
<td>0.85</td>
<td>0.86</td>
<td>0.84</td>
<td>0.87</td>
<td>0.84</td>
</tr>
<tr>
<td>Manzanillo</td>
<td>(2.5 mg/L SeNPs)</td>
<td>0.73</td>
<td>0.84</td>
<td>0.85</td>
<td>0.85</td>
<td>0.86</td>
<td>0.84</td>
<td>0.87</td>
<td>0.85</td>
</tr>
<tr>
<td>Manzanillo</td>
<td>(10 mg/L Ag NPs)</td>
<td>0.79</td>
<td>0.81</td>
<td>0.85</td>
<td>0.85</td>
<td>0.86</td>
<td>0.84</td>
<td>0.87</td>
<td>0.85</td>
</tr>
<tr>
<td>Koroneiki</td>
<td>(control)</td>
<td>0.74</td>
<td>0.79</td>
<td>0.83</td>
<td>0.87</td>
<td>0.86</td>
<td>0.83</td>
<td>0.87</td>
<td>0.84</td>
</tr>
<tr>
<td>Koroneiki</td>
<td>(2.5 mg/L SeNPs)</td>
<td>0.74</td>
<td>0.86</td>
<td>0.86</td>
<td>0.86</td>
<td>0.87</td>
<td>0.86</td>
<td>0.87</td>
<td>0.87</td>
</tr>
<tr>
<td>Koroneiki</td>
<td>(5 mg/L SeNPs)</td>
<td>0.74</td>
<td>0.82</td>
<td>0.84</td>
<td>0.84</td>
<td>0.85</td>
<td>0.84</td>
<td>0.86</td>
<td>0.85</td>
</tr>
<tr>
<td>Koroneiki</td>
<td>(5 mg/L Ag NPs)</td>
<td>0.79</td>
<td>0.81</td>
<td>0.79</td>
<td>0.84</td>
<td>0.87</td>
<td>0.84</td>
<td>0.87</td>
<td>0.86</td>
</tr>
<tr>
<td>Koroneiki</td>
<td>(10 mg/L Ag NPs)</td>
<td>0.80</td>
<td>0.80</td>
<td>0.78</td>
<td>0.83</td>
<td>0.86</td>
<td>0.85</td>
<td>0.87</td>
<td>0.86</td>
</tr>
</tbody>
</table>

NPs= Nanoparticles.
TiO$_2$, carbon nanotubes and fullerenes, metal oxide NPs which inhibited growth of the plants (Rodriguez et al., 2011; Srivastava et al., 2015). Several studies have demonstrated the phytotoxicity of NPs applied at higher concentrations to plant tissue (Craig and Jason, 2011; Ghodake et al., 2011). Ewais et al. (2015) reported that the addition of AgNPs to tissue culture medium stimulates changes in the callus morphology and anatomy and alter DNA and protein profiles. Moreover, López-Moreno et al. (2010) observed damage in the genomic DNA structure in soybean plants due to treatment with CeO$_2$ NPs by RAPD-PCR assay.

**Cluster analysis**

The genetic distances (GDs) among three olive cultivars treated with NPs and the control ranged from 0.72 to 0.94 based on combined RAPD and DAMD profiles. The cluster analysis using UPGMA based on genetic distances of markers found that the three olive cultivars could be divided into three major clusters (fig. 4). These results were in agreement with Hassan et al. (2017) found that the genetic distances (GDs) among three olive cultivars regenerated in vitro and the donor plants ranged from 0.74 and 0.94 depending on RAPD-PCR analysis.

The first cluster (I): (similarity range of 0.72 to 0.94) composed of ‘Picual’ (P) treated with 2.5 mg/L SeNPs and 10 mg/L AgNPs, ‘Koroneiki’ (K) (2.5 and 5 mg/L SeNPs and 5 and 10 mg/L AgNPs) and ‘Manzanillo’ (M) (the control). The second cluster (II): (similarity range of 0.73 to 0.92) involved into ‘Picual’ (P) treated with 5 mg/L SeNPs and 5 mg/L AgNPs, ‘Manzanillo’ (M) treated at (2.5 and 5 mg/L SeNPs and 5 and 10 mg/L AgNPs) and ‘Koroneiki’ (K) control. The third cluster (III): (similarity range of 0.73 to 0.82 similarity) consisted of ‘Picual’ (P) control (table 5 and fig. 4). The cluster analysis is one of the important ways in numerical analysis concerning fragment recording and analysis of RAPD fingerprinting. In this study, cluster analysis was done to evaluate the level of DNA polymorphism between the NPs treated and untreated olive plants. A dendrogram was constructed using the distance matrix by using UPGMA method observed that olive plantlets treated with high concentrations of selenium and silver NPs induced changes on DNA level depending on the NPs concentration.

**Conclusion**

This study is the first report stated that 5 mg L$^{-1}$ AgNPs had a positive effect on in vitro performance. However, 10 mg L$^{-1}$ AgNPs and 2.5 and 5 mg L$^{-1}$ SeNPs induced changes on genomic DNA level. In addition, RAPD and DAMD assays can be successfully applied as a sensitive method of detecting DNA stability and genotoxicity in olive plants treated with different concentrations of selenium and silver NPs.

**References**


Ghosh, M., M. Bandyopadhyay and A. Mukherjee (2010).
Genotoxicity of titanium dioxide (TiO₂) nanoparticles at two trophic levels: plant and human lymphocytes. *Chemosphere, 81*: 1253-1262.


